Results from a phase I/II trial of cusatuzumab combined with azacitidine in patients with newly diagnosed acute myeloid leukemia who are ineligible for intensive chemotherapy

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Abstract

Cusatuzumab is a high-affinity, anti-CD70 monoclonal antibody under investigation in acute myeloid leukemia (AML). This two-part, open-label, multicenter, phase I/II trial evaluated cusatuzumab plus azacitidine in patients with newly diagnosed AML ineligible for intensive chemotherapy. Patients received a single dose of cusatuzumab at one of four dose levels (1, 3, 10, or 20 mg/kg) 14 days before starting combination therapy. In phase I dose escalation, cusatuzumab was then administered on days 3 and 17, in combination with azacitidine (75 mg/m^2) on days 1-7, every 28 days. The primary objective in phase I was to determine the recommended phase II dose (RP2D) of cusatuzumab plus azacitidine. The primary objective in phase II was efficacy at the RP2D (selected as 10 mg/kg). Thirty-eight patients were enrolled: 12 in phase I (three per dose level; four with European LeukemiaNet 2017 adverse risk) and 26 in phase II (21 with adverse risk). An objective response (≥partial remission) was achieved by 19/38 patients (including 8/26 in phase II); 14/38 achieved complete remission. Eleven patients (37.9%) achieved an objective response among the 29 patients in phase I and phase II treated at the RP2D. At a median follow-up of 10.9 months, median duration of first response was 4.5 months and median overall survival was 11.5 months. The most common treatment-emergent adverse events were infections (84.2%) and hematologic toxicities (78.9%). Seven patients (18.4%) reported infusion-related reactions, including two with grade 3 events. Thus, cusatuzumab/azacitidine appears generally well tolerated and shows preliminary efficacy in this setting. Investigation of cusatuzumab combined with current standard-of-care therapy, comprising venetoclax and azacitidine, is ongoing.

Introduction

Intensive induction and consolidation chemotherapy with curative intent is recommended for patients with newly diagnosed acute myeloid leukemia (AML), provided they demonstrate adequate drug tolerance.[1] For patients unsuitable for intensive chemotherapy, standard of care is evolving. Hypomethylating agents (HMA), such as azacitidine and decitabine, have been central to treatment for several years.[1-4] However, since the start of this phase I/II trial, other agents have been studied in combination with HMA, and recent data have established venetoclax plus an HMA as a new standard of care in this setting.[5] Despite this changing landscape, overall survival (OS) is <15 months with venetoclax/azacitidine, and even in the subgroup of responding patients, median duration of response is <18 months,[5] indicating a need for more effective therapies. Acute myeloid leukemia is driven by leukemic stem cells (LSC) that have a key role in initiating and sustaining malignancy.[6] LSC also have a capacity for self-renewal and their persistence is believed to be the primary cause of relapse in AML.[7-9] Selective elimination of LSC, without affecting normal hematopoiesis, is challenging owing to the greater resistance of LSC to conventional chemotherapy compared with more differentiated AML blasts.[10,11] CD70, a tumor necrosis factor receptor ligand, is a very promising target due to its consistent expression on LSC and AML blasts.[12,13] In AML, the binding of CD70 to its receptor, CD27, on LSC and subsequent downstream signaling activates gene-expression profiles that promote LSC proliferation, reduce differentiation, and lead to release of soluble CD27 (sCD27).[12,13] Serum sCD27 levels are increased in patients with newly diagnosed AML,[12,13] and are a strong, independent negative predictor of cancer prognosis.[12]

Cusatuzumab (ARGX-110) is a high-affinity, anti-CD70 monoclonal antibody that blocks CD70/CD27 signaling, leading to inhibition of LSC proliferation, a reduction in leukemic blast cells, blockade of regulatory T-cell survival (preventing tumor immune escape), and restoration of normal myeloid differentiation.[12-15] It also exerts direct Fc-mediated, effector functions via enhanced antibody-dependent cellular cytotoxicity (modified using POTELLIGENT® Technology), complement-dependent cytotoxicity, and antibody-dependent cellular phagocytosis, leading to apoptosis of leukemic cells and blasts.[13,14]

Treatment with HMA upregulates CD70 expression on LSC isolated from patients with newly diagnosed AML, and combined anti-CD70 and HMA treatment can synergistically decrease LSC to a greater extent than blocking CD70 alone.[13] Hence, there is a rationale for studying cusatuzumab in combination with an HMA.

First-in-human studies have shown that single-agent cusatuzumab is well tolerated and is biologically active in patients with advanced solid tumors or hematologic malignancies, including AML.[16,17] Promising early responses and pharmacodynamic activity at all cusatuzumab doses were demonstrated in interim data from a two-part, phase I/II dose-escalation and expansion study undertaken to investigate the potential of cusatuzumab in combination with azacitidine for the treatment of newly diagnosed patients with AML who were not candidates for intensive chemotherapy.[13] Treatment was also well tolerated without reaching a maximum tolerated dose. This work builds on the interim data from the same study, reporting results for the entire study population, including the phase II expansion.

Methods

Study design

This was an open-label, multicenter, non-randomized, dose-escalation (phase I) and expansion (phase II) study. Phase I employed a 3+3 design with dose increments based on a modified Fibonacci scheme. Phase I enrolled patients in four sequential dose cohorts (1, 3, 10, 20 mg/kg). In each cohort, patients received a single intravenous (IV) dose of cusatuzumab on day -14 followed by combination therapy, comprising IV cusatuzumab on days 3 and 17 plus azacitidine 75 mg/m^2 subcutaneously or IV on days 1-7, every 28 days. To mitigate infusion-related reactions (IRR), all patients were premedicated with acetaminophen, an antihistamine, and an IV glucocorticoid prior to cusatuzumab infusion. The first patient in each cohort was monitored until cycle 1 day 7; if no dose-limiting toxicities (DLT) occurred (*Online Supplementary Appendix*), further patients were enrolled in the cohort. Subsequent cohorts were opened upon approval from the Data Safety Monitoring Board. Phase II patients received cusatuzumab at the recommended phase II dose (RP2D) from phase I plus azacitidine at the same dose/schedule as phase I. Patients were treated for as long as they derived clinical benefit or until disease progression, unacceptable toxicity, death, withdrawal, or loss to follow-up.

The study was carried out in accordance with the principles of the Declaration of Helsinki, Good Clinical Practice guidelines, and regulatory and country-specific requirements, and is registered with clinicaltrials.gov (NCT03030612). The protocol was approved by an independent ethics committee/review board. Patients gave written informed consent.

Eligibility

Adults (≥18 years) with newly diagnosed AML (defined by a blast count of ≥20%), unsuitable for intensive chemotherapy, were enrolled. Additional eligibility criteria included an expected life expectancy of ≥3 months and Eastern Cooperative Oncology Group (ECOG) performance status of 0-2. Patients with any prior chemotherapy/radiotherapy for AML

(except hydroxyurea/hydroxycarbamide, which had to be discontinued prior to the first day of azacitidine administration) were excluded. Full eligibility criteria are listed in the *Online Supplementary Appendix*.

Endpoints and assessments

In phase I, the primary endpoint was incidence of DLT at each dose of cusatuzumab plus standard dose azacitidine (to inform RP2D). In phase II, the primary endpoint was overall response rate (ORR), defined as complete remission (CR) plus CR with incomplete recovery (CRi) plus morphologic leukemia-free state plus partial remission at cusatuzumab dose established in phase I plus standard dose azacitidine. Secondary endpoints in both parts included: treatment-emergent adverse events (TEAE); pharmacokinetics and immunogenicity of cusatuzumab in peripheral blood; minimal residual disease evaluation by multiparameter flow cytometry performed in one of two laboratories (see *Online Supplementary Appendix*); time to, level, and duration of response; OS; 30/60-day mortality; and transfusion independence. Pharmacodynamic markers were also assessed (see *Online Supplementary Appendix*).

Response evaluation by investigators was based on established criteria (*Online Supplementary Table S1*). Safety and tolerability were assessed throughout; evaluations included TEAE (graded using National Cancer Institute-Common Terminology Criteria for Adverse Events; NCI-CTCAE, version 4.03), laboratory parameters, electrocardiogram, vital signs, physical examinations, and ECOG performance status (*Online Supplementary Appendix*).

Statistical analysis

Using Simon's two-stage design with a target ORR of 50% *versus* 25%, 5% type 1 error, and 20% type 2 error, 24 patients were needed in phase II for 80% power. The null hypothesis was to be rejected if the ORR was >37.5% (>9/24 responses). Analyses of the primary endpoint were performed on the full analysis set (patients who received an infusion of any study drug), as well as a combination therapy analysis set (patients from phase I and phase II who received cusatuzumab at the RP2D and azacitidine). Statistical inference according to the Simon's design was based on the full analysis set as well as the combination therapy analysis set. Time-to-event data were analyzed by Kaplan-Meier methods.

Results

Patients' cohorts, treatment and response

Between January 2017 and February 2019, 38 patients were enrolled at eight sites across Switzerland, France, and Italy, and treated in the phase I dose escalation (n=12) or phase II expansion (n=26). The dataset used for this analysis includes extended follow-up for 12 phase I patients treated at the 1, 3, 10, and 20 mg/kg dose levels (three per cohort) and 26 phase II patients treated at the RP2D of 10 mg/kg. The data cut-off for this analysis was July 1, 2020.

Treatment was discontinued in 34 out of 38 patients (89.5%): 2/3 patients at 1 mg/kg, 3/3 at 3 mg/kg, 26/29 at 10 mg/kg, and 3/3 at 20 mg/kg. Reasons for treatment discontinuation were: progressive disease (n=17, 50%), adverse event (AE) (n=6, 17.6%), death (n=6, 17.6%), investigator decision (n=2, 5.9%), protocol deviation (n=1, 2.9%), withdrawal of consent (n=1, 2.9%), and other (n=1, 2.9%; patient wished to proceed to allogeneic transplant).

Recommended phase II dose

The 10 mg/kg dose level of cusatuzumab was selected as the RP2D based on a prespecified interim analysis of phase I data for the 1-10 mg/kg dose cohorts in April 2018. No DLT were observed in any of these dose cohorts and the maximum tolerated dose was not reached. At the time of the interim analysis, data for the DLT period were incomplete for two out of three patients in the phase I 20 mg/kg dose cohort. None of the three patients treated at 20 mg/kg went on to experience DLT.

Patients' baseline characteristics

Baseline demographics and disease characteristics are shown in Table 1. Across all patients, median age was 75 years (range, 59-90), 50% of patients were female, 7.9% had an ECOG performance status of 2, 34.2% had secondary AML, and 65.8% had adverse genetic risk per European LeukemiaNet (ELN) 2017 criteria[3] (with risk categories assigned *post hoc* by an independent reviewer). Median time from diagnosis to treatment was 14.5 days (range, 3-139).

Efficacy

Response data are presented in Table 2 and Figure 1. An objective response was achieved by 19/38 patients in the full analysis set (both study phases combined), for an ORR of 50% (95% confidence interval [95%CI]: 33.4-66.6). All responding patients achieved CR or CRi (no partial remission or morphologic leukemia-free state): 14 (36.8%) with CR and five (13.2%) with CRi. In phase II, 8/26 patients responded to treatment at 10 mg/kg (ORR, 30.8%; 95%CI: 14.3-51.8), including five with CR (19.2%) and three with CRi (11.5%). A response of CR/CRi was achieved by 4/4 patients with favorable ELN risk status, 7/9 with intermediate status, and 8/25 with adverse status (*Online Supplementary Table S2*). Of the 19 patients with a best response of CR/CRi, six (31.6%) achieved minimal residual disease negativity.

Among patients in phase I and phase II receiving cusatuzumab 10 mg/kg, 11/29 (37.9%) achieved an objective response in the full analysis set. Two of these patients were classified as non-evaluable because they died in the

Table 1. Patients' demographics and baseline characteristics.

Characteristic	Phase I					Phase II	Total (N=38)
	1 mg/kg (N=3)	3 mg/kg (N=3)	10 mg/kg (N=3)	20 mg/kg (N=3)	All doses (N=12)	10 mg/kg (N=26)	
Age in years, median (range)	77.0 (75-81)	71.0 (71-84)	74.0 (64-75)	76.0 (72-77)	75.0 (64-84)	75.5 (59-90)	75.0 (59-90)
Sex, N (%)							
Female	1 (33.3)	2 (66.7)	1 (33.3)	1 (33.3)	5 (41.7)	14 (53.8)	19 (50)
Male	2 (66.7)	1 (33.3)	2 (66.7)	2 (66.7)	7 (58.3)	12 (46.2)	19 (50)
Race, N (%)							
White	3 (100)	3 (100)	3 (100)	3 (100)	12 (100)	19 (73.1)	31 (81.6)
Not reported	0	0	0	0	0	7 (26.9)	7 (18.4)
ECOG performance status, N (%)							
0	1 (33.3)	3 (100)	0	0	4 (33.3)	9 (34.6)	13 (34.2)
1	2 (66.7)	0	3 (100)	3 (100)	8 (66.7)	14 (53.8)	22 (57.9)
2	0	0	0	0	0	3 (11.5)	3 (7.9)
AML type, N (%)							
De novo	0	1 (33.3)	3 (100)	2 (66.7)	6 (50)	19 (73.1)	25 (65.8)
Secondary	3 (100)	2 (66.7)	0	1 (33.3)	6 (50)	7 (26.9)	13 (34.2)
Genetic risk category per ELN 2017 criteria, N (%)							
Favorable	0	1 (33.3)	0	1 (33.3)	2 (16.7)	2 (7.7)	4 (10.5)
Intermediate	2 (66.7)	2 (66.7)	2 (66.7)	0	6 (50)	3 (11.5)	9 (23.7)
Adverse	1 (33.3)	0	1 (33.3)	2 (66.7)	4 (33.3)	21 (80.8)	25 (65.8)
Time from diagnosis to treatment, days, median (range)	28.0 (3-64)	29.0 (13-69)	8.0 (3-17)	30.0 (6-47)	22.5 (3-69)	13.5 (6-139)	14.5 (3-139)

AML: acute myeloid leukemia; ECOG: Eastern Cooperative Oncology Group; ELN: European LeukemiaNet.

Table 2. Best response to cusatuzumab plus azacitidine.

	Phase I					Phase II	Phase I+II		Total (N=38)
	1 mg/kg (N=3)	3 mg/kg (N=3)	10 mg/kg (N=3)	20 mg/kg (N=3)	All doses (N=12)	10 mg/kg (N=26)	10 mg/kg (N=29)	10 mg/kg (N=27)[a]	
ORR,[b] N (%) [95%CI]	3 (100) [29.2-100]	2 (66.7) [9.4-99.2]	3 (100) [29.2-100]	3 (100) [29.2-100]	11 (91.7) [61.5-99.8]	8 (30.8) [14.3-51.8]	11 (37.9) [20.7-57.7]	11 (40.7) [22.4-61.2]	19 (50) [33.4-66.6]
Response category, N (%)									
CR	2 (66.7)	2 (66.7)	2 (66.7)	3 (100)	9 (75)	5 (19.2)	7 (24.1)	7 (25.9)	14 (36.8)
CRi	1 (33.3)	0	1 (33.3)	0	2 (16.7)	3 (11.5)	4 (13.8)	4 (14.8)	5 (13.2)
MLFS	0	0	0	0	0	0	0	0	0
PR	0	0	0	0	0	0	0	0	0
SD[c]	0	1 (33.3)	0	0	1 (8.3)	16 (61.5)	16 (55.2)	16 (59.3)	17 (44.7)
NE	0	0	0	0	0	2 (7.7)	2 (6.9)	0	2 (5.3)

CI: confidence interval; CR: complete remission; CRi: complete remission with incomplete recovery; MLFS: morphologic leukemia-free state; NE: not evaluable; ORR: overall response rate; PR: partial remission; SD: stable disease. [a]Excluding 2 patients who did not receive azacitidine and died before first post-treatment disease assessment. [b]Overall response includes patients with a response of CR, CRi, MLFS, or PR. [c]Treatment failure responses were categorized as SD.

interval after their first dose of cusatuzumab and did not receive either azacitidine nor their first post-treatment assessment on cycle 1 day 1; these deaths were unrelated to cusatuzumab. Excluding these two patients resulted in the combination therapy analysis set and an objective response rate of 11/27 (40.7%). While the total number of patients in

these analyses deviates from the original Simon's design of 24, *ad hoc* Simon's criteria based on the same design parameters calls for rejecting the null hypothesis if ORR is ≥12/29 or ≥11/27. The null hypothesis is not rejected in the full analysis set but is rejected in the combination therapy analysis set.

For the full analysis set, at a median follow-up of 10.6 months (range, 0.3-38.2), median time to first response was 3.2 months (range, 0.5-12.4) and median duration of first response was 4.5 months (range, 0.02-33.7). Median OS was 11.5 months (95%CI: 7.3-17.1), with a 12-month OS rate of 49%.

Independence from red blood cell (RBC) and platelet (PLT) transfusion (defined as reaching ≥8 consecutive weeks without a transfusion from administration of the first dose of study drug) was observed in 24 patients (63.2%). Twenty-four patients (63.2%) achieved RBC transfusion independence and 29 (76.3%) obtained PLT transfusion independence. Median duration of RBC/PLT independence was 13.0 months (range, 2.0-37.9).

Safety and tolerability

Median duration of study treatment was 5.8 months (range, 0-37.9) and a median of six cycles were administered (range, 1-40).

All 38 patients had ≥1 TEAE and all experienced a grade ≥3 TEAE (Table 3). The most common TEAE were febrile neutropenia and neutropenia (n=15 each, 39.5%), followed by anemia, thrombocytopenia, pneumonia, and pyrexia (n=14 each, 36.8%). After pneumonia, the next most frequent infectious TEAE were sepsis (n=11, 28.9%) and urinary tract infection (n=4, 10.5%). Thirty-two patients (84.2%) had a

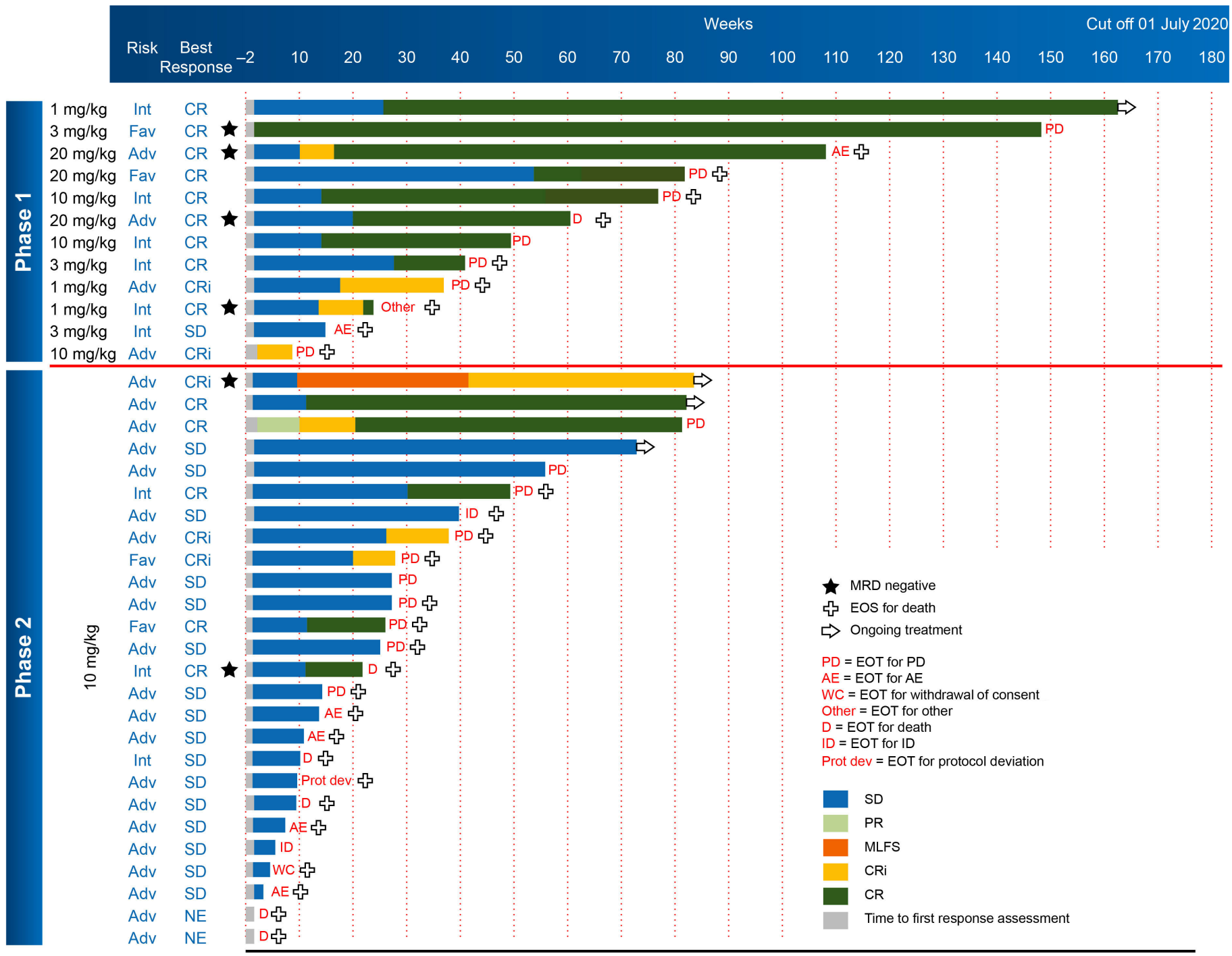

Figure 1. Swimmer plot illustrating responses and outcomes in patients with newly diagnosed acute myeloid leukemia treated with cusatuzumab plus azacitidine (total study population, N=38). Timing of death is not included. Adv: adverse; AE: adverse event; CR: complete remission; CRi: complete remission with incomplete recovery; EOS: end of study; EOT: end of treatment; Fav: favorable; ID: investigator decision; Int: intermediate; MLFS: morphologic leukemia-free state; MRD: minimal residual disease; NE: not evaluable; PD: progressive disease; PR: partial remission; SD: stable disease.

serious TEAE which led to hospitalization in all but one of these patients (Table 3). The most common serious TEAE were febrile neutropenia (n=13, 34.2%), sepsis (n=11, 28.9%), and pneumonia (n=10, 26.3%) (*Online Supplementary Table S3*). TEAE leading to discontinuation of any study agent were reported in eight patients (21.1%; n=6 at 10 mg/kg, and n=1 each at 3 and 20 mg/kg) and included anal abscess, diverticulitis, pneumonia, general deterioration in physical health, multiple organ dysfunction syndrome, cardiac failure, hypopituitarism, enterocolitis, and hypertension (all n=1). There were 10 fatal TEAE (26.3%; eight at 10 mg/kg and two at 20 mg/kg); none were considered drug-related. TEAE leading to death were: multiple organ dysfunction syndrome (n=3), general deterioration in physical health

Table 3. Summary of treatment-emergent adverse events following treatment with cusatuzumab plus azacitidine.

Patients with ≥1 TEAE,[a] N (%)	**Dose group**								**Total (N=38)**	
	1 mg/kg (N=3)		**3 mg/kg (N=3)**		**10 mg/kg (N=29)**		**20 mg/kg (N=3)**			
Any TEAE, N (%) Grade ≥3 Drug-related	3 (100) 3 (100) 3 (100)		3 (100) 3 (100) 3 (100)		29 (100) 29 (100) 27 (93.1)		3 (100) 3 (100) 3 (100)		38 (100) 38 (100) 36 (94.7)	
Serious TEAE, N (%) Grade ≥3 Leading to hospitalization	3 (100) 3 (100) 3 (100)		3 (100) 3 (100) 3 (100)		24 (82.8) 24 (82.8) 23 (79.3)		2 (66.7) 2 (66.7) 2 (66.7)		32 (84.2) 32 (84.2) 31 (81.6)	
TEAE leading to any study drug discontinuation, N (%)	0		1 (33.3)		6 (20.7)		1 (33.3)		8 (21.1)	
TEAE leading to death, N (%) Drug-related	0 0		0 0		8 (27.6) 0		2 (66.7) 0		10 (26.3) 0	
Most common TEAE (≥15% of all patients),[b] N (%)	**All**	**Gr ≥3**	**All**	**Gr ≥3**	**All**	**Gr ≥3**	**All**	**Gr ≥3**	**All**	**Gr ≥3**
Febrile neutropenia	2 (66.7)	2 (66.7)	1 (33.3)	1 (33.3)	10 (34.5)	10 (34.5)	2 (66.7)	2 (66.7)	15 (39.5)	15 (39.5)
Neutropenia	1 (33.3)	1 (33.3)	3 (100)	3 (100)	9 (31)	9 (31)	2 (66.7)	2 (66.7)	15 (39.5)	15 (39.5)
Anemia	1 (33.3)	1 (33.3)	3 (100)	3 (100)	10 (34.5)	10 (34.5)	0	0	14 (36.8)	14 (36.8)
Thrombocytopenia	2 (66.7)	2 (66.7)	3 (100)	3 (100)	7 (24.1)	7 (24.1)	2 (66.7)	1 (33.3)	14 (36.8)	13 (34.2)
Pneumonia[c]	2 (66.7)	1 (33.3)	0	0	10 (34.5)	6 (20.7)	2 (66.7)	2 (66.7)	14 (36.8)	9 (23.7)
Pyrexia	2 (66.7)	0	2 (66.7)	1 (33.3)	9 (31.0)	0	1 (33.3)	0	14 (36.8)	1 (2.6)
Constipation	1 (33.3)	0	1 (33.3)	1 (33.3)	7 (24.1)	0	2 (66.7)	0	11 (28.9)	1 (2.6)
Nausea	1 (33.3)	0	0	0	9 (31.0)	0	1 (33.3)	0	11 (28.9)	0
Sepsis[d]	0	0	0	0	10 (34.5)	10 (34.5)	1 (33.3)	1 (33.3)	11 (28.9)	11 (28.9)
Vomiting	0	0	2 (66.7)	0	5 (17.2)	0	2 (66.7)	0	9 (23.7)	0
Leukopenia	2 (66.7)	2 (66.7)	2 (66.7)	1 (33.3)	2 (6.9)	2 (6.9)	1 (33.3)	0	7 (18.4)	5 (13.2)
Diarrhea	0	0	0	0	4 (13.8)	0	3 (100)	1 (33.3)	7 (18.4)	1 (2.6)
Chills	1 (33.3)	0	1 (33.3)	0	4 (13.8)	1 (3.4)	0	0	6 (15.8)	1 (2.6)
Cough	1 (33.3)	0	2 (66.7)	0	3 (10.3)	0	0	0	6 (15.8)	0
Hypokalemia	0	0	1 (33.3)	1 (33.3)	5 (17.2)	0	0	0	6 (15.8)	1 (2.6)

Gr: grade. [a] Treatment-emergent adverse events (TEAE) are defined as AE with onset or worsening on or after the date of the first dose of study treatment up to and including 30 days after date of last dose of study medication. [b] TEAE are listed in decreasing frequency of any-grade TEAE in the total study population (N=38). [c] Pneumonia includes the following preferred terms: pneumonia, bacterial pneumonia, and fungal pneumonia. [d]Sepsis includes the following preferred terms: *Enterobacter sepsis*, *Escherichia sepsis*, pseudomonal bacteremia, sepsis, septic shock, and *Staphylococcal* bacteremia.

(n=2), and pneumonia, sepsis, acute coronary syndrome, large intestine perforation, and respiratory failure (n=1 each).

Seven patients (18.4%) reported IRR, of which chills (n=5, 13.2%) and pyrexia (n=2, 5.3%) were the most common. Two grade 3 IRR (5.3%; chills n=1 and hypertension n=1) were observed; the hypertension event led to treatment discontinuation. There were no IRR observed in the 20 mg/kg cohort.

Two deaths (5.3%; both due to an AE) occurred within 30 days. Four (10.5%) deaths occurred within 60 days of first treatment with cusatuzumab, all in the phase II 10 mg/kg cohort: three due to an AE (n=2 multiple organ dysfunction syndrome, n=1 pneumonia), one due to other reasons (assisted-suicide).

Pharmacokinetics and pharmacodynamics

In phase I, after IV administration of the monotherapy dose (on day -14) or second dose (on cycle 1 day 3, post-azacitidine) of cusatuzumab, mean maximum serum concentration (C_{max}) and mean area under the serum concentration-time curve from time 0 to 14 days (AUC_{14d}) increased with increasing doses (*Online Supplementary Table S4*). Mean serum half-life ($t_{1/2}$) ranged from 6.1 to 10.4 days across the four dose cohorts in phase I. There was no obvious change in dose-normalized parameters with increasing dose, suggesting exposure increased in an approximately dose-proportional manner over the dose range 1-20 mg/kg. In phase II, after IV administration of the 10 mg/kg monotherapy dose of cusatuzumab, mean C_{max} was 195 μg/mL; AUC_{14d} was 32,932 μg.h/mL, and $t_{1/2}$ was 11.1 days. After administration of the second dose on cycle 1 day 3, mean C_{max} was 233 μg/mL; AUC_{14d} was 38,298 μg.h/mL, and $t_{1/2}$ was 8.3 days.

There was a lower median percentage bone marrow blast count from baseline (screening) to cycle 1 day 1, i.e., following the single monotherapy dose of cusatuzumab, and prior to the first dose of azacitidine and second dose of cusatuzumab (*Online Supplementary Figure S1*). Analysis of pharmacodynamic markers showed most patients exhibited the biggest decrease in sCD27 levels after the initial cusatuzumab monotherapy dose (*Online Supplementary Figure S2*). Finally, expression of CD70 on the blasts was confirmed by flow cytometry but could not be associated with clinical response (*Online Supplementary Figure S3*).

Immunogenicity

Among 36 cusatuzumab-treated patients with evaluable samples, 11 (30.6%) tested positive for antibodies to cusatuzumab post dose: 10/11 patients had antibodies first detected in cycle 1; one of 11 patients had antibodies first detected in cycle 3. One of four patients with a positive sample at baseline became positive for treatment-boosted antidrug antibodies. The small sample size means that no conclusions as to how cusatuzumab concentration affects immunogenicity can be reached.

Discussion

This study assessed the feasibility of combining the anti-CD70 monoclonal antibody, cusatuzumab, with standard-dose azacitidine in patients with newly diagnosed AML who were ineligible to receive intensive chemotherapy due to advanced age, comorbidities, and/or a poor performance status. Building on the interim results of the phase I dose-escalation period of this study,[13] we found that half of the 38 patients (50%) treated with cusatuzumab/azacitidine achieved an objective response (CR or CRi). For the full analysis set of all patients who received the cusatuzumab 10 mg/kg treatment from phase I and phase II (n=29), the null hypothesis is not rejected; it is, however, rejected in the combination therapy analysis set (n=27) after excluding two patients who died before receiving combination therapy. It should be noted that after the data lock for this study, another patient who had been treated with cusatuzumab 10 mg/kg had a confirmed CRi, which would allow for the null hypothesis to be rejected for both the full analysis and combination therapy sets.

While these responses clearly demonstrate the clinical activity of the combination, response rates in the phase II part were lower than those reported in the initial phase I interim analysis, where high response rates were reported.[13] The apparent discrepancy between the phase I interim data and final combined results may be explained, at least in part, by the small number of patients in the two phases of the study and by differences in baseline characteristics, particularly the prevalence of adverse genetic risk per ELN criteria (33.3% in phase I *vs.* 80.8% in phase II), with higher response rates among favorable- and intermediate-risk patients (11/13 for favorable/intermediate risk compared with 8/25 for adverse risk). Despite the lower than anticipated response rates, durable CR were observed in a number of patients at all dose levels, including at the 10 mg/kg dose of cusatuzumab selected for expansion, and almost two-thirds of patients (63.2%) achieved transfusion independence, which is a strong prognostic factor in unfit patients with AML.[18] Responses were also observed in each ELN 2017 genetic risk group (a good predictor of prognosis in newly diagnosed AML[3,19]), indicating the feasibility of the combination for all patients, including those with adverse risk profiles. Notably, the median OS of 11.5 months compares favorably with a recent real-world report for azacitidine alone (7.1 months),[20] suggesting that the cusatuzumab/azacitidine combination is worthy of further study.

Cusatuzumab combined with azacitidine was generally well tolerated, with most TEAE consistent with those expected for an AML population undergoing treatment with azaciti-

dine,[4,21] and there was no obvious dose dependency for toxicities. The most common TEAE were infections and hematologic toxicities, which were generally manageable. IRR, a common side effect of many monoclonal antibodies used to treat hematologic malignancies,[22-24] were the only notable addition to the AE profile. These reactions were usually mild or moderate in intensity and were generally managed successfully by interrupting the cusatuzumab infusion, providing specific treatment for the symptom manifested, and restarting the infusion at a reduced rate.

Formation of antidrug antibodies have previously been shown to contribute to loss of efficacy;[25] however, the clinical impact of the antidrug antibodies observed in this cohort remains uncertain since neutralizing assays were not performed. The pharmacodynamic data were consistent with previous assessments for cusatuzumab and support its mechanism of action to reduce AML blasts and decrease serum sCD27 levels.[12-14,16] CD70 expression could be detected on baseline peripheral blood blasts but could not be identified as a predictor of response to cusatuzumab/azacitidine in patients with newly diagnosed AML as observed for other immune-related molecules.[26,27] These data suggest that CD70 expression is not a limiting factor for the efficacy of cusatuzumab treatment. In addition, it has been shown that HMA treatment upregulates CD70.[13]

The pharmacokinetic evaluations also supported other prior investigations[16,17] and showed that cusatuzumab exposure increases in an approximately dose-proportional manner following treatment over the dosing interval 1-20 mg/kg. This approximate dose-proportional increase in systemic exposure, combined with the (limited) response and safety data seen at the 20 mg/kg dose level, provides a rationale for further investigating the higher dose of cusatuzumab. Though the cohort was small, all three patients treated at 20 mg/kg, including two with adverse genetic risk per ELN, achieved CR without evidence of disease progression after >1 year of therapy. There were also no indications of additional toxicity. As these data only became available after the RP2D of 10 mg/kg had been selected, the optimal dose of cusatuzumab for further study remains uncertain. Consequently, the ongoing randomized, phase II, CULMINATE trial is evaluating the efficacy and safety of the 10 and 20 mg/kg doses of cusatuzumab combined with azacitidine in a similar AML study population.[28] The clinical potential of cusatuzumab is also being investigated in combination with the new standard of care, venetoclax, with or without azacitidine (clinicaltrials.gov NCT04150887). This latter trial is informed by preclinical data showing that cusatuzumab works synergistically with both azacitidine and venetoclax to eliminate primary human AML LSC.[29]

In conclusion, our findings suggest that the combination of cusatuzumab and azacitidine is generally well tolerated and may be efficacious in patients with previously untreated AML not eligible for intensive chemotherapy. Studies are ongoing to establish the optimal dose level of cusatuzumab (10 *vs.* 20 mg/kg) plus azacitidine and assess the feasibility of combining cusatuzumab with venetoclax, with or without azacitidine.

Disclosures

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(PETHEMA study group). CRi is listed as inventor on a patent held by the University of Bern on targeting CD70 for treatment in AML. PS is a Janssen R&D employee. JT has stock or stock options from JNJ Company 401K, JNJ Stock Option Plan. AFO has received support for the present manuscript (funding) from SAKK, Rising Tide, Gateway; grants or contracts from argenx (funding); royalties and licenses from argenx/Janssen; patent: Targeting CD70 in myeloid leukemia. TP, LA and UB have no conflicts of interest to disclose.

Contributions

TP and AO contributed to study and protocol design, conduct of the study, and interpretation of results. CRi and UB contributed to study and protocol design, and interpretation of results. MB, RM, NV, LA, CRe, CP and GG helped conduct the study. DG and AH contributed to study protocol and interpretation of results. SF provided consultancy services to argenx and contributed to interpretation of results. AJ, XM, KN, LX, JT and PS were involved in interpretation of results. All authors critically reviewed and revised the manuscript, and approved the final version for submission.

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Data-sharing statement

Janssen has an agreement with the Yale Open Data Access (YODA) Project to serve as the independent review panel for evaluation of requests for clinical study reports and participant-level data from investigators and physicians for scientific research that will advance medical knowledge and public health. Data will be made available following publication and approval by YODA of any formal requests with a defined analysis plan. For more information on this process, or to make a request, please visit The Yoda Project site at http://yoda.yale.edu. The data-sharing policy of Janssen Pharmaceutical Companies of Johnson & Johnson is available at https://www.janssen.com/clinical-trials/transparency.

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